Dissociation Between Ionic Remodeling and Ability to Sustain Atrial Fibrillation During Recovery From Experimental Congestive Heart Failure

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Background—Congestive heart failure (CHF) downregulates atrial transient outward ($I_{to}$), slow delayed rectifier ($I_{Kr}$), and $L$-type $Ca^{2+}$ ($I_{Ca,L}$) currents and upregulates $Na^+$$-$$Ca^{2+}$ exchange current ($I_{NCX}$) (ionic remodeling) and causes atrial fibrosis (structural remodeling). The relative importance of ionic versus structural remodeling in CHF-related atrial fibrillation (AF) is controversial.

Methods and Results—We measured hemodynamic and echocardiographic parameters, mean duration of burst pacing–induced AF (DAF), and atrial-myocyte ionic currents in dogs with CHF induced by 2-week ventricular tachypacing (240 bpm), CHF dogs allowed to recover without pacing for 4 weeks (REC), and unpaced controls. Left ventricular ejection fraction averaged $58.6\pm1.2\%$ (control), $36.2\pm2.3\%$ (CHF, $P<0.01$), and $57.9\pm1.6\%$ (REC), indicating full hemodynamic recovery. Similarly, left atrial pressures were $2.2\pm0.3$ (control), $13.1\pm1.5$ (CHF), and $2.4\pm0.4$ (REC) mm Hg. CHF reduced $I_{to}$ density by $\approx65\%$ ($P<0.01$), decreased $I_{Ca,L}$ density by $\approx50\%$ ($P<0.01$), and diminished $I_{Kr}$ density by $\approx40\%$ ($P<0.01$) while increasing $I_{NCX}$ density by $\approx110\%$ ($P<0.05$). In REC, all ionic current densities returned to control values. DAF increased in CHF (1132 versus 143.2 seconds, control) and remained increased with REC (1014.2 seconds). Atrial fibrous tissue content also increased in CHF (2.1$\pm0.2\%$ for control versus 10.2$\pm0.7\%$ for CHF, $P<0.01$), with no recovery observed in REC (9.4$\pm0.8\%$, $P<0.01$ versus control, $P=\text{NS}$ versus CHF).

Conclusions—With reversal of CHF, there is complete recovery of ionic remodeling, but the prolonged-AF substrate and structural remodeling remain. This suggests that structural, not ionic, remodeling is the primary contributor to AF maintenance in experimental CHF. (Circulation. 2004;109:412-418.)

Key Words: heart failure $\bullet$ ion channels $\bullet$ atrium $\bullet$ fibrillation

Congestive heart failure (CHF) is a common cause of atrial fibrillation (AF). Mechanisms underlying AF associated with CHF are incompletely understood. Ventricular tachypacing–induced CHF increases the ability to sustain AF in dogs. CHF produces substantial atrial fibrosis (structural remodeling) and associated local conduction slowing, which may play a major role in promoting AF. Conversely, CHF also causes atrial ionic remodeling, downregulating $I_{to}$, $I_{Kr}$, and $I_{Ca,L}$ and upregulating $Na^+$$-$$Ca^{2+}$ exchanger current ($I_{NCX}$). $I_{NCX}$ can carry depolarizing currents after phase 3 repolarization, inducing delayed afterdepolarizations (DADs). Increased $I_{NCX}$ promotes DAD-associated ventricular tachyarrhythmias in a rabbit CHF model, and atrial tachyarrhythmias have been associated with DADs in the canine ventricular-tachypacing model of CHF. Thus, both ionic and structural remodeling induced by CHF could contribute to associated AF, and their relative importance is unclear.

We have found that the hemodynamic changes and chamber dilation caused by 5 weeks of ventricular tachypacing recover completely on cessation of tachypacing, but fibrosis persists, along with a substrate that can maintain AF. We reasoned that with full hemodynamic recovery, there might be recovery from CHF-induced atrial ionic remodeling, allowing for a dissociation between CHF-related structural and ionic remodeling; however, we were unable to identify information in the literature on changes in atrial ionic remodeling after recovery from CHF. The present study was designed to assess changes in atrial ionic currents produced by 2 weeks of ventricular tachypacing and to compare them with changes in dogs subjected to 2 weeks of tachypacing followed by 4 weeks in sinus rhythm to allow for recovery from CHF. Ionic-current changes were related to alterations in hemodynamics, cardiac chamber size, and function (assessed echocardiographically); atrial fibrosis; and the duration of burst pacing–induced AF (DAF).

Methods

Animal Model

Animal care procedures followed Canadian Council on Animal Care guidelines and were approved by the Animal Research Ethics Board.

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Committee of the Montreal Heart Institute. Mongrel dogs were assigned to 3 groups: unpaced control dogs (CTL, n=30; mean weight, 25.1±0.9 kg), 2-week ventricular tachypacing–induced CHF dogs (n=21; 24.9±1.2 kg), and 2-week ventricular tachypacing followed by 4-week recovery dogs (REC, n=20; 24.5±0.5 kg). Among the control dogs, 3 were sham operated (pacemaker not activated) and maintained for the full 6-week REC protocol. Because their electrophysiological, hemodynamic, and AF properties were indistinguishable from noninstrumented acute controls, the results of all control dogs were analyzed together.

Dogs in the sham, CHF, and REC groups were initially anesthetized with diazepam (0.25 mg/kg IV) and ketamine (5.0 mg/kg IV) halothane (1% to 2%). Unipolar leads were inserted fluoroscopically into the right ventricular apex and connected to a pacemaker implanted in the neck. After 24-hour recovery, the ventricular pacemaker was programmed to capture the right ventricle at 240 bpm for 2 weeks. CHF dogs were subjected to open-chest study at 2 weeks, whereas the pacemaker was inactivated for the subsequent 4 weeks in REC dogs, followed by open-chest study. In vivo studies were performed in the first 16 control, 16 CHF, and 10 REC dogs. Because performing both in vivo and in vitro studies in each dog greatly prolonged the experiments, subsequent dogs were used for in vitro study only, after verifying that in vitro results were similar whether or not they were preceded by in vivo study.

In Vivo Studies

On open-chest study days, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV load, 29.25 mg·kg⁻¹·h⁻¹ infusion), and median sternotomy was performed. Effective refractory period (ERP) was measured at the left atrial (LA) appendage with 15 basic (S₁) stimuli, followed by a premature (S₂) stimulus applied with 5-ms decrements (ERP = longest S₂S₂ failing to capture). All stimuli were twice-threshold, 2-ms pulses. The mean of 3 ERP values at each basic cycle length was used for analysis. AF was induced by burst pacing (10-Hz, 2-ms pulses, 4 times threshold current). AF was defined as atrial rhythm >400 bpm, with irregular electrogram morphology and rate. After ERP measurement, mean DAF in each dog was determined on the basis of 10 inductions from 58.6±0.9 to 77.3±1.2%. When the chest was opened, moderate to severe pericardial effusions and pulmonary congestion were evident in all CHF dogs but no control or REC dogs. Ventricular and arterial pressures were increased (Table). REC dogs were hemodynamically indistinguishable from noninstrumented acute controls, the results of which are presented as previously described.8 Hemodynamic data were obtained with fluid-filled catheters and disposable pressure transducers. Closed-chest 2D echocardiographic studies were performed in apical 4-chamber, apical 2-chamber, and parasternal long-axis views in sinus rhythm at baseline, after 2-week tachypacing, and after 4 weeks of recovery in REC dogs. Care was taken to maintain the same plane of imaging for each study. After open-chest study, 6 atria of control, 5 atria of CHF, and 6 atria of REC dogs were immersed in 10% neutral-buffered formalin. Tissue samples obtained from 4 LA regions were stained with Masson's trichrome, and microscopic images were analyzed with SigmaScan 4.0 (Jandell Scientific).

Cellular Electrophysiology

Currents were recorded with whole-cell patch clamp at 36±0.5°C. Borosilicate glass electrodes had tip resistances between 1.5 and 3.0 MΩ when filled. Compensated series resistance and capacitive time constants averaged 3.1±0.9 MΩ and 247±7 ms. Cell capacitance averaged 87±5 pF for CTL, 111±7 pF for CHF, and 91±4 pF for REC myocytes (n=90 per group, P<0.01 for CHF versus CTL and REC). To control for cell-size variability, currents are expressed as densities (pA/pF). Junction potentials between bath and pipette solutions averaged 9.7±0.7 mV and were not compensated.

Solutions

The solution for cell-storage contained (in mmol/L) KCl 20, KH₂PO₄ 10, dextrose 10, mannitol 40, L-glutamic acid 70, β-OH-butyric acid 10, taurine 20, and EGTA 10 and 0.1% BSA (pH 7.3, KOH). Tyrode’s (extracellular) solution contained (mmol/L) NaCl 136, KCl 5.4, MgCl₂ 1, CaCl₂ 1, NaH₂PO₄ 0.33, HEPES 5, and dextrose 10 (pH 7.35, NaOH). For delayed rectifier current recording, nifedipine (5 μmol/L), 4-aminopyridine (2 mmol/L), and atropine (200 nmol/L) were added to suppress L-type calcium current (Iₘ,L). transient outward current (Iₘ,O), and 4-aminopyridine–dependent muscarinic K⁺ currents.26 Dofetilide (1 μmol/L) was added for Iₘ,O recording. For Iₘ,C recording, nifedipine was replaced by CdCl₂ (200 μmol/L). Na⁺ current (Iₘ,C) contamination was avoided by use of a holding potential of −50 mV or by substitution of equimolar Tris-HCl for NaCl. The internal solution for K⁺ current recording contained (mmol/L) K-aspartate 110, KCl 20, MgCl₂ 1, MgATP 5, LiGTP 0.1, HEPES 10, Na-phosphocreatinine 5, and EGTA 5.0 (pH 7.3, KOH).

The external solution for Iₘ,L recording contained (in mmol/L) tetracyclammonium-Cl 136, CsCl 5.4, CaCl₂ 2, MgCl₂ 0.8, HEPES 10, and dextrose 10 (pH 7.4, CsOH). Niflumic acid (50 μmol/L) was added to inhibit Ca²⁺–dependent Cl⁻ current.23 The internal solution for Iₘ,C recording was (in mmol/L) CsCl 120, TEA-Cl 20, MgCl₂ 1, MgATP 5, LiGTP 0.1, EGTA 10, and HEPES 10 (pH 7.3, CsOH). Iₘ,C was recorded as previously described,3,11 with extracellular solution containing (in mmol/L) NaCl 140, CaCl₂ 2, MgCl₂ 1, dextrose 10, HEPES 10, and nifedipine 5 μmol/L and internal solution containing CsCl 130, NaCl 5, MgATP 4, and HEPES 10 (pH 7.3, CsOH).

Statistics

Nonlinear curve fitting was performed with Clampfit in pCLAMP6. Group data are presented as mean±SEM. Statistical comparisons were by ANOVA and t tests with Bonferroni’s correction. A 2-tailed value of P<0.05 was taken to indicate statistical significance.

Results

Hemodynamic and Echocardiographic Indices

When the chest was opened, moderate to severe pericardial effusions and pulmonary congestion were evident in all CHF dogs but no control or REC dogs. Ventricular and arterial systolic pressures were reduced in CHF dogs, and left atrial systolic (LV) end-diastolic, LA, and right atrial (RA) pressures were increased (Table). REC dogs were hemodynamically indistinguishable from controls.

The evolution of echocardiographic parameters in REC dogs is illustrated in Figure 1. LV ejection fraction decreased from 58.6±1.2% (baseline) to 36.2±2.3% after 2 weeks of ventricular tachypacing and returned to 57.9±1.6% (P=NS versus CTL) over the subsequent 4 weeks without tachypacing. LA systolic and diastolic areas increased significantly after 2-week tachypacing, by 45.1±4.4% and 77.3±7.4%, respectively, with full recovery 4 weeks later. Similarly, LA...
fractional shortening was reduced 38.3±3.2% by 2 weeks of tachypacing, with subsequent full recovery.

Ionic Remodeling

Transient Outward Current
Typical $I_o$ in control cells is shown in Figure 2A. Mean $I_o$ density was significantly reduced by CHF (Figure 2B), eg, at +40 mV, from 6.5±0.7 (CTL) to 2.5±0.3 pA/pF. In REC cells, $I_o$ was not significantly altered compared with control (eg, 6.6±1.1 pA/pF at +40 mV). The reduced $I_o$ density in the CHF group was not associated with a changed $I_o$-voltage relationship, as shown by the normalized data in Figure 2C.

Activation voltage dependence of $I_o$ was assessed from data obtained with the protocol shown in Figure 2, A and B, on the basis of the relationship $I_o=I_{max}(V-V_r)(G/G_{max})$, where $I_o$ and $G$ are current and conductance at voltage $V$, $I_{max}$ and $G_{max}$ are maximum current and conductance, and $V_r$ is reversal potential. $V_r$ as evaluated by tail currents after 2.2-ms depolarizations to +50 mV, averaged −71.5±1.7, −69±3.0, and −69.8±2.1 mV (without junction-potential correction) in CTL, CHF, and REC, respectively (6 cells per group, $P=NS$). No significant shifts in activation voltage dependence were seen (Figure 2D). Activation $V_{1/2}$ based on Boltzmann fits to data in each experiment averaged +10.3±2.5 mV in CTL, +11.9±2.5 mV in CHF, and +11.8±1.1 mV in REC (10 cells per group, $P=NS$). Corresponding slope factors were 11.5±0.8, 12.4±0.3, and 11.0±0.6 mV ($P=NS$). Inactivation voltage dependence was studied with 1000-ms prepulses from a holding potential of −70 mV, followed by 200-ms test pulses to +50 mV.

Typical $I_{Ca,L}$ recordings from a control cell are shown in Figure 3A. $I_{Ca,L}$ density decreased significantly (Figure 3B) in CHF myocytes and recovered in REC myocytes (eg, at +10 mV, from −8.9±0.9 nA/pF in control to −4.5±0.7 nA/pF in CHF and −7.9±0.5 nA/pF in REC, $n=15, 10, and 18$ cells, respectively). The voltage dependence of $I_{Ca,L}$ activation and inactivation was unaffected by CHF or REC (Figure 3C). $V_r$ (determined by linear extrapolation of the ascending I-V limb to the voltage-axis) was not different in the CTL, CHF, and REC groups (63.6±1.4, 62.6±1.6, and 64.0±1.3 mV, respectively, $n=10$ cells per group). Activation $V_{1/2}$ averaged −4.6±0.7, −5.0±1.1, and −5.2±0.7 mV in the CTL, CHF, and REC groups, respectively, $n=10$ cells per group ($P=NS$), and slope factors were 4.6±0.2, 4.5±0.2, and 4.7±0.2 mV ($P=NS$, Figure 3C). $I_{Ca,L}$ recovery time constants were not different among groups: $34.1±3.6$ ms in CTL, $32.9±5.2$ ms (CHF), and $30.4±2.7$ ms (REC, $n=6$ cells per group, $P=NS$; Figure 3D). $I_{Ca,L}$ frequency dependence, as evaluated by 15 pulses from −80 to +10 mV at 1 to 6 Hz, was also similar for all groups (Figure 3E). $I_{Ca,L}$ inactivation kinetics were biexponential, with no differences among groups (Figure 3F).

Slow Delayed Rectifier Current

$I_{Ks}$ recordings from a control cell are shown in Figure 4A. CHF cells showed significantly reduced tail (Figure 4B) and step (Figure 4C) current densities compared with control or REC. Voltage dependence of $I_{Ks}$ activation
(tail-current analysis, Figure 4D) was not altered by CHF and REC (V<sub>i0</sub>, 21.0±2.6, 19.2±1.5, and 19.7±1.2 mV in the CTL, CHF, and REC groups, respectively, n=12 cells per group). I<sub>Ks</sub> activation kinetics at +40 mV were biexponential, with a slow-phase time constant averaging 2280±376, 2100±287, and 2380±272 ms in the control, CHF, and REC groups, respectively (P=NS, n=10 cells per group). The fast-phase time constant was similarly unchanged, averaging 260±25, 242±24, and 264±47 ms in the control, CHF, and REC groups (P=NS, n=10 cells per group).

Na<sup>+</sup>-Ca<sup>2+</sup> Exchange Current
I<sub>Ncx</sub> recordings in CTL, CHF, and REC cells are shown in Figure 5, A–C. After 5-ms depolarizations from a holding potential of −70 mV, I<sub>Ncx</sub> was recorded during 100-ms hyperpolarizing pulses. Substitution of Li<sup>+</sup> for extracellular Na<sup>+</sup> suppressed inward currents, as expected for I<sub>Ncx</sub>.<sup>3,11</sup> CHF significantly increased I<sub>Ncx</sub> density (Figure 5D), with a return to control values in REC cells (P=NS, n=10 cells per group).

In Vivo Electrophysiology and Tissue Histopathology
CHF significantly increased atrial ERP at all basic cycle lengths, a change that reversed with recovery (Figure 6A), consistent with the reversal of ionic remodeling seen in REC cells for all currents studied. DAF was substantially increased in CHF dogs (Figure 6B). Despite full hemodynamic and echocardiographic recovery in REC dogs, DAF remained as prolonged as in CHF dogs. AF cycle length averaged 96±2 ms in control dogs and increased to 114±2 ms (P<0.001) in CHF dogs, returning to 99±3 ms (P=NS versus CTL) in REC dogs. The shorter AF cycle length, tracking ERP at short cycle lengths, and similar DAF in REC dogs versus CHF suggest that ERP prolongation may not be a significant factor in AF promotion.

We previously showed that 5 weeks of ventricular tachypacing–induced CHF result in atrial structural remodeling that fails to recover during 5 weeks without tachypacing. To determine whether similar findings occur after 2 weeks of tachypacing, as in the present study, we performed histopathologic analyses, the results of which are shown in Figure 7. In CHF and REC dogs, there was extensive interstitial fibrosis, which was not present in control dogs. Mean data indicate that fibrous tissue content was signifi-
cantly increased for CHF and REC dogs in all LA regions. Overall, fibrous tissue content averaged 2.1±0.2% for control dogs, versus 10.2±0.7% for CHF and 9.4±0.8% for REC dogs (P<0.01 for each versus CHF, P=NS for CHF versus REC).

Discussion
We have shown that 2 weeks of rapid ventricular pacing causes signs of CHF, along with ionic remodeling that includes decreased I_{K_s}, I_{to}, and I_{Ca,L} and increased I_{NCX}. Ionic remodeling recovers fully, as do hemodynamic and echocardiographic indices of CHF, 4 weeks after cessation of tachypacing. However, neither the substrate for AF (as assessed by DAF during open-chest study) nor structural remodeling (as assessed by atrial histopathology) shows a significant return toward control values despite full hemodynamic recovery.

Relationship to Previous Studies of AF Mechanisms in Experimental Models
Atrial tachycardia for >24 hours causes ionic remodeling that decreases atrial ERP and plays an important role in the increased ability of tachycardia-remodeled atria to sustain AF. CHF produces important atrial fibrosis, which is associated with localized conduction abnormalities that are believed to promote AF by stabilizing reentry. However, CHF also produces substantial atrial ionic remodeling. Among other changes, CHF increases expression of I_{NCX}, an important carrier of postrepolarization transient inward currents that cause DADs. DADs can induce triggered activity, and evidence for a role of DADs in atrial tachyarrhythmias in the canine CHF model has been presented by Stambler et al. Thus, it is quite conceivable that CHF-induced atrial ionic remodeling could play an important role in the associated AF substrate. The present study indicates that atrial ionic remodeling is not a necessary condition for prolonged AF in the CHF-associated substrate, because DAF was just as prolonged in REC dogs as in CHF dogs, despite full recovery of atrial ionic remodeling and CHF after 4 weeks without tachypacing.

As in our findings, Stambler et al noted recovery of hemodynamic abnormalities, LA diameter, and ERP changes when ventricular tachypacing was discontinued for 7 to 14 days, after a mean 20-day ventricular tachypacing period in dogs. Despite this significant recovery, there was no improvement in the inducibility of sustained atrial tachyarrhythmias.
Ionic Remodeling in Cardiac Disease and Its Reversal

Ionic remodeling is a feature of a variety of cardiac diseases and has important relevance for therapeutic approaches.14 The downregulation of $I_{\text{to}}$, $I_{\text{K}}$, and $I_{\text{Ca}}$ and upregulation of $I_{\text{NCX}}$ that we observed are similar to previous findings in atrial myocytes after 5 weeks of ventricular tachypacing and to a variety of reports at the ventricular level in models of cardiac failure.3,15 Our finding that ionic remodeling reverses when the initiating stimulus is removed is consistent with observations that CHF-induced changes in the properties and regulation of ventricular $I_{\text{Ca}}$ were reversed by hemodynamic support in patients placed on LV assist devices.16 These findings support the plasticity of ionic remodeling and a need to consider changes in ion channel function in the development and use of electrophysiologically active drug therapy.

Potential Limitations

We chose to use LA myocytes for the patch-clamp experiments in this study, because in previous work, we have found LA remodeling associated with CHF to be more intense than that in the RA.2 The use of LA myocytes may explain why the ionic changes we observed after 2 weeks of ventricular tachypacing in the present experiments were comparable to those in RA cardiomyocytes after 5 weeks of tachypacing in...
a previous study. The results might have been quantitatively different had we studied cells from a different atrial region; however, the qualitative similarity in ionic changes between the present results and previous findings suggests similar types of ionic changes in response to CHF in different atrial regions.

We elected to focus on atrial ionic currents shown to change with CHF in previous work. We therefore studied $I_{\text{Na}}$, $I_{\text{Ks}}$, $I_{\text{Ca,L}}$, and $I_{\text{NCX}}$. The goal of the present study was to assess whether ionic currents that are altered by CHF recover with hemodynamic improvement; therefore, we judged that little additional information could be gained by recording such currents as atrial rapid and ultrarapid delayed rectifier currents and T-type Ca$^{2+}$ current, which are unaffected in dogs with CHF produced by 5 weeks of ventricular tachypacing. In addition, changes in $I_{\text{Na}}$ and cell coupling and such structural factors as nerve sprouting and gap-junction distribution, which we did not study, could play a role.

In the present study, we evaluated only the substrate for AF maintenance; we did not evaluate potential triggers in the form of atrial ectopy or characteristics of AF induction in CHF and recovery. Previously, we found that under ketamine/diazepam/isoflurane anesthesia, a decrease in DAF occurs with recovery from CHF; ionic remodeling may therefore contribute to AF maintenance under this form of anesthesia. We are not suggesting that our results imply that ionic remodeling is irrelevant to AF promotion by CHF but simply that it is not an essential condition, because prolonged AF can be induced under morphine/chloralose anesthesia during recovery from CHF in the absence of ionic remodeling.

**Conclusions**

Extensive atrial ionic remodeling occurs after 2 weeks of ventricular tachypacing, in association with atrial fibrosis, hemodynamic and echocardiographic indicators of CHF, and the ability to induce prolonged AF. When tachypacing is then discontinued for 4 weeks, ionic remodeling is fully reversed. However, the ability to support prolonged AF and atrial fibrosis remains unchanged, suggesting that structural and not ionic remodeling is the primary basis for prolonged AF in this experimental model of CHF.

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